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Executive Summary

Descriptor 9 (*Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards*) is one of eleven Marine Strategy Framework Directive (MSFD) qualitative Descriptors to be used in determining whether Good Environmental Status (GES) has been achieved for European regional seas (Directive EC/2008/56)**.** For monitoring of compliance with GES for Descriptor 9, contaminant concentrations in fish and seafood should be compared against the EC regulatory levels. A sampling programme targeting appropriate fish species was recently designed for Descriptor 9. This design was based around existing fish stock assessment research vessel surveys, with fish sampled from each trawling location with a probability proportional to the landings by the Scottish fishing fleet. Haddock, monk and herring were selected based on their importance to the human diet (based on fish landings) and to represent different groups of fish (e.g. high trophic level, high fat content). Using this sampling design, samples were collected in 2013 and 2014 and the muscle tissue analysed for polychlorinated biphenyls (PCBs) and trace metals. PCBs were mainly below detection limits in monkfish and haddock, but above detection limits in herring where concentrations for the ICES6 CBs ranged from < LoD (in one sample only) to 17.5 µg kg^{-1} wet weight. For metals, Cd and Pb were mainly below detection limits, whereas Hg was detected in all samples. Concentrations of Hg were higher in monkfish than in haddock and herring. Although monkfish has a low lipid content, it is at the highest trophic level of the three species. Cd, Hg, Pb and the ICES6 PCB concentrations were below the regulatory levels in all samples. The 95^{th} percentile of the distributions of the trace metal and ICES6 PCBs concentrations were estimated for each species and area and compared against the regulatory limits. All were significantly below the regulatory levels, except for mercury in monkfish from the west coast.

Introduction

The Marine Strategy Framework Directive (MSFD) Descriptor 9 (Contaminants in Food) states that ***'contaminants in fish and other sea food for human consumption do not exceed levels established by Community legislation or other relevant standards***'. Therefore, for Descriptor 9 assessment, contaminant concentrations in fish and seafood should be compared against the EC regulatory levels[1]. European regulatory levels (EC/1881/2006, as amended, and EC/1259/2011) are available for trace metals (Cd, Hg and Pb), chlorinated dioxins and furans[i], dioxin-like polychlorinated biphenyls (DL-PCBs)[ii] and non DL-PCBs[iii] (ICES6 PCBs) in fish muscle, crustacea and bivalve molluscs; regulatory levels are also available for polycyclic aromatic hydrocarbons (PAHs; indicated by benzo[*a*]pyrene) in crustacea and bivalves and for dioxins (including DL-PCBs) and non DL-PCBs in fish liver (Table 1). The fish and shellfish contaminant monitoring currently undertaken in Scotland was reviewed in 2014 with respect to the requirements of the Marine Strategy Framework Directive (MSFD)[2]. Contaminant data from Scottish shellfish monitoring programmes, mainly undertaken by the Scottish Environment Protection Agency (SEPA) and the Food Standards Agency in Scotland (FSAS[iv]), could be used for Descriptor 9 assessments, although currently FSAS data are not submitted to the UK Marine Environment Monitoring and Assessment National database (MERMAN). Marine Scotland Science (MSS) monitor contaminants (including Cd, Hg, Pb and PCBs) in fish at sites around Scotland under the UK Clean Seas Environment Monitoring Programme (CSEMP), in order to satisfy the requirements of the Convention for the Protection of the Marine Environment of the North East Atlantic (OSPAR) and of MSFD Descriptor 8. Most of these data are unsuitable for Descriptor 9 assessments as commercially exploited

[i] Dioxins (sum of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), expressed as World Health Organisation (WHO) toxic equivalent using the WHO-toxic equivalency factors (WHO-TEFs).

[ii] Of the 209 PCB congeners, the most toxic are the so-called 'dioxin-like' PCBs (DL-PCBs). The DL-PCBs are the four non-*ortho* (CB77, 81, 126, and 169) and eight mono-*ortho* (CB105, 114, 118, 123, 156, 157, 167, and 189) congeners. DL-PCBs are stereo-chemically similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and, therefore, have similar toxic and biological responses to those of dioxins.

[iii] The seven ICES (International Council for the Exploration of the Sea) PCBs (CB28, 52, 101, 118, 153, 138, and 180) were recommended for monitoring by the European Union Community Bureau of Reference; these congeners were selected as indicators of wider PCB contamination due to their relatively high concentrations in technical mixtures and their wide chlorination range (3-7 chlorine atoms per molecule). The ICES 7 PCBs have been part of the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP) since 1998. MPCs have been set for the ICES 6 (excludes CB118 which is classed as a DL-PCB).

[iv] Superseded by Food Standards Scotland since 1 April 2015.

fish species/size-ranges are not targeted and, with the exception of metals, contaminants are measured in fish liver rather than in the edible flesh.

Due to the limited amount of Scottish contaminant data in fish collected for environmental monitoring programmes that are suitable for the assessment of Good Environmental Status (GES) against Descriptor 9, a fish sampling programme was designed for Descriptor 9. This is described in detail in Webster *et al.*[2]. The target species selected for the Descriptor 9 sampling programme in both the North Sea and West of Scotland were haddock (high landings, high trophic level, limited migration, lean), herring (high landings, moderate trophic level, moderate migration, high lipid content), and monk (moderate landings, high trophic level, limited migration, lean).

Sampling was based around existing research vessel surveys for obtaining indices of abundance for use in fish stock assessments. MSS conducts annual bottom trawl surveys in the North Sea (OSPAR Region II) in quarter 1 (Q1: January – March) and quarter 3 (Q3: July – September). These surveys provide data that are used in the assessment of fish stocks in ICES Area IV. There are also annual bottom trawl surveys to the West of Scotland (OSPAR Region III) in Q1 and quarter 4 (Q4: October – December) that provide abundance indices for ICES Division VIa. Division VIa extends beyond Scottish waters but, for simplicity, is regarded as the sampling area for Descriptor 9. The herring acoustic survey also covers part of the North Sea and the West of Scotland in Q3. Fish were sampled on these surveys from haul locations with probabilities proportional to landings.

Regulatory levels are currently available for PCBs (ICES6 PCBs), chlorinated dioxins and furans, DL-PCBs and trace metals in fish. MSS have accredited methods for the analysis of PCBs (including the ICES6 PCBs and DL-PCBs) and trace metals in fish, but do not have the capability to measure dioxins. Dioxin (and DL-PCB) concentrations will be much lower than concentrations of the ICES6 PCBs. Until recently Commission Regulation (EC) No 252/2012 required the application of gas chromatography coupled with high resolution mass spectrometry (GC-HRMS) as the confirmatory method for the quantification of dioxins and furans[v]. Neither Marine Scotland nor SEPA have GC-HRMS and, therefore, do not have the capability to undertake this analysis, which would have to be outsourced at high cost. However, papers have been published looking at alternative methods to predict the total toxic equivalent (TEQ) concentration (for dioxins and 'dioxin-like' CBs) in fish tissue, using total or indicator PCB concentrations. Therefore, dioxin TEQs may be estimated

[v]This regulation has recently been updated (Commission Regulation EC/589/2014) and now allows for the use of triple quadrupole gas chromatography mass spectrometry (GC-MS/MS) for the quantitative and confirmatory analysis of dioxins.

from the PCB concentrations using published models and could be used to demonstrate if dioxin TEQs are likely to exceed the maximum permitted concentration (MPC). All fish (edible muscle tissue only) were analysed for PCBs and trace metals at MSS. The results of these analyses are described in this report.

Sample Collection

Fish were collected by the research vessel MRV *Scotia* on annual bottom trawl surveys to the West of Scotland and the North Sea (Figure 1). The design of this sampling programme has previously been described[2]. In the North Sea, haddock were collected during the 2014 Q1 bottom trawl survey and monkfish and herring during the 2014 Q3 bottom trawl survey. To the West of Scotland, haddock were collected during both the 2013 Q4 and 2014 Q1 bottom trawl surveys (the Q4 survey could not be completed due to bad weather). Monkfish were collected during the 2014 Q1 bottom trawl survey and herring during the 2014 Q3 herring acoustic survey.

At the time of capture, all whole fish samples were wrapped separately in aluminium foil and stored at -20 ±5^{o}C. On return to the laboratory, the fish muscle tissue was removed, homogenised and subsamples taken for the analysis of PCBs and trace metals.

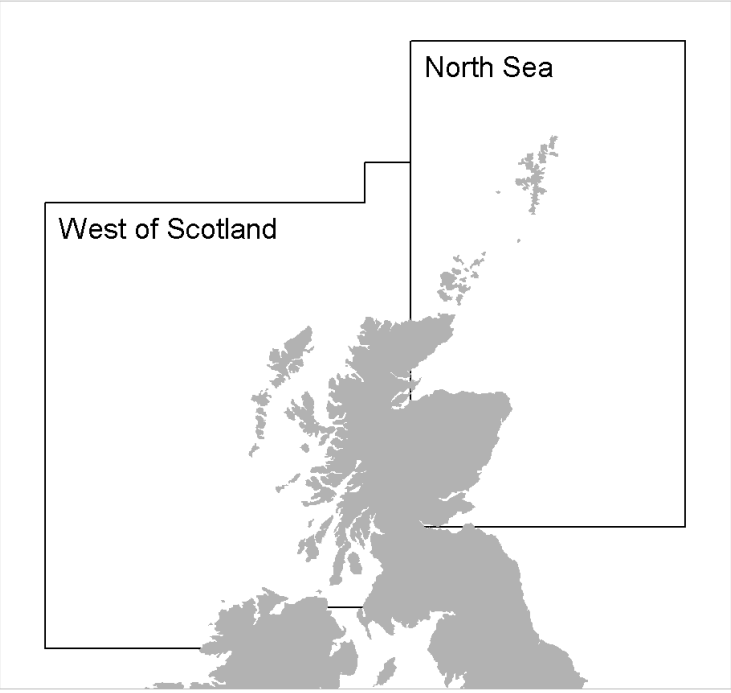

Figure 1: North Sea and West of Scotland D9 sampling areas.

Lipid Determination

The total lipid content was determined according to the method of Smedes[3]. The biota sample (fish muscle, 2-5 g) was weighed into a centrifuge tube and *iso*-propanol (18 ml) and cyclohexane (20 ml) added. The sample was homogenised, then de-ionised water (~13 – 22 ml, depending on the moisture content of the sample) was added and the mixture homogenised. Centrifugation was used to separate the organic extract from the particulate material. A second extraction was carried out with 13% (v/v) *iso*-propanol in cyclohexane. The two extracts were combined and the solvent removed by rotary evaporation before drying in an oven at 80^{o}C (± 5) for one hour. The weight of residue was determined and the lipid content calculated as % wet weight.

Determination of Polychlorinated Biphenyls (PCBs)

Pressurised Liquid Extraction (PLE)

PCBs were extracted as described by Webster *et al.*[4,5]. Briefly, samples were mixed with sodium sulphate and spiked with appropriate internal standards (PCBs: ^{13}C-CB28, ^{13}C-CB52, ^{13}C-CB101, ^{13}C-CB153, ^{13}C-CB138, ^{13}C-CB156, ^{13}C-CB180, ^{13}C-CB189, ^{13}C-CB194 and ^{13}C-CB209) prior to pressurised liquid extraction (PLE). Solvent washed PLE cells (100 ml) were packed as follows: solvent washed filter paper, pre-washed sodium sulphate (10 g), 5% deactivated alumina (30 g), solvent washed filter paper and the biota/sodium sulphate mixture prepared as above. Samples were extracted by PLE using an ASE 300 (Dionex Ltd., Camberley, Surrey, UK) at a temperature of 100^{o}C and a pressure of 1,500 psi. The extraction solvent was *iso*-hexane.

Following PLE, the extract was concentrated by Syncore (fitted with flush-back module) to ~ 0.5 ml and passed through silica columns. The concentrated extracts were analysed for PCBs by gas chromatography (GC) - electron impact mass spectrometry (EIMS).

Determination of Polychlorinated Biphenyls (PCBs) by Gas Chromatography – Electron Impact Mass Spectrometry (GC-EIMS)

The concentration and composition of 28 *ortho*-substituted CB congeners (CB31, 28, 52, 44, 49, 70, 74, 110, 101, 99, 97, 149, 118, 132, 153, 105, 157, 137, 138, 158, 183, 128, 156, 180, 187, 189, 170, 194) were determined by GC-MS in electron impact mode using an HP6890 Series gas chromatograph interfaced with an

HP5975 MSD, fitted with a cool, on-column injector and a 50 m x 0.22 mm x 25 µm SGE HT-8 column (SGE, Milton Keynes, UK). Temperature programmes have previously been described (Webster *et al.*, 2009 and 2011a).

Determination of Trace Metals in Fish Flesh

For the determination of metals (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Ba, Hg, and Pb), homogenised fish flesh (~0.6 g wet weight) was digested using 2 ml of hydrogen peroxide (Trace Analysis grade, Fisher Chemical, Loughborough, UK) and 5 ml of nitric acid (Aristar-grade, VWR, Lutterworth, UK) in sealed Teflon vessels using a Multiwave 3000 digestion system (Anton Paar, St Albans, UK) and the digests diluted with ultra-pure (18.2MΩ.cm) water. Concentrations were determined using fully traceable calibration standards (Inorganic Ventures, Christiansburg, VA, USA) by Inductively Coupled Plasma Mass Spectrometry (ICPMS; Agilent 7700x, Agilent, Stockport, UK) equipped with on-line addition of internal standards (Ge, Rh, Bi, Ir), Micro-mist™ nebuliser and Peltier-cooled, modified, Scott spray chamber. To remove polyatomic interferences, concentrations of most elements were determined in collision cell mode using He gas at a flow rate of 4.3 ml/min (4.7 ml/min for Se); Ag, Ba, Hg and Pb were determined in no-gas mode.

Quality Control

The lipid, PCB and trace metal methods are accredited by the United Kingdom Accreditation Service (UKAS) to ISO 17025. All methods were validated by the replicate analysis of standards and samples, and through spiking experiments or analysis of certified reference materials (CRMs). Limits of detection (LoDs) were determined through the repeat analysis (n=7-10) of a low concentration sample and the LoD calculated from 4.65 x standard deviation (SD) of the mean concentration. LoDs were dependent on the sample size. The replicate analysis of standards on separate days gave coefficient of variation (CV%) of ~ 3% for PCBs analysed by GC-MS. Recoveries of between 75% and 110% were achieved for PCB spiked biota and CRMs. LoDs were, around 0.05 µg kg^{-1} wet weight for fish muscle samples (2-5 g). Metal recoveries on DORM-3 CRM varied between 92% (Fe) and 112% (Hg), between-batch CRM reproducibility varied from 4.2% (Cd) to 13% (Ni), $HORWITZ_R$ ratios were better than 2 (except for Ni, Fe (both 2.7) and Ag: 4.4) and detection limits for the key elements of Cd, Hg and Pb were 4.8, 3.2 and 13.6 µg kg^{-1} wet weight, respectively.

Internal quality control procedures incorporated the use of a Laboratory Reference Material (LRM) for PCBs and lipid, and DORM-3 a Certified Reference Material

(CRM; NRC, Canada) for trace metals, in each batch of samples. Procedural blanks were performed with each batch of samples, and the final concentrations adjusted accordingly. The data obtained from the LRM and CRM were transferred onto NWA Quality Analyst and Shewhart charts were produced with warning and action limits being drawn at ± 2 x and ± 3 x the standard deviation of the mean, respectively. CRM data was accepted if recoveries were between 80 and 120% of the certified concentration. Quality assurance was further demonstrated through successful participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) Laboratory Performance Studies.

Table 1

Regulatory limits on the maximum permitted concentrations (wet weight) of certain environmental contaminants in edible portions of fish and shellfish (whole fish if appropriate). TEQ = Toxic Equivalent Concentration (summed concentrations of certain planar organic compounds based upon their relative toxicity[6]).

Regulation	Compound or element	Maximum permitted concentration	Species to which the limit applies
EC/1881/2006	Pb	0.3 mg kg^{-1}	Fish and shellfish with the main exceptions indicated below:
EC/1881/2006	Pb	0.5 mg kg^{-1}	Crustacea (excluding crab brown meat & head / thorax of lobster)
EC/1881/2006	Pb	1.0 mg kg^{-1}	Cephalopods (without viscera)
EC/1881/2006	Pb	1.5 mg kg^{-1}	Bivalve molluscs
EC/629/2008	Cd	0.05 mg kg^{-1}	Fish and shellfish with the exceptions indicated below:
EC/629/2008	Cd	0.1 mg kg^{-1}	Bonito, common two-banded seabream, eel, grey mullet, horse mackerel or scad (*Trachurus sp.*), louvar or luvar, sardine, sardinops, tuna, wedge sole.
EC/629/2008	Cd	0.2 mg kg^{-1}	Bullet tuna
EC/629/2008	Cd	0.3 mg kg^{-1}	Anchovy, swordfish
EC/629/2008	Cd	0.5 mg kg^{-1}	Crustacea (excluding crab brown meat & head / thorax of lobster and similar large crustaceans)
EC/629/2008	Cd	1.0 mg kg^{-1}	Cephalopods (without viscera), bivalve molluscs
EC/1881/2006	Hg	0.5 mg kg^{-1}	Fish and shellfish with the exceptions of crab brown meat, head / thorax meat of lobster (and similar spp.) and the species indicated below:
EC/629/2008	Hg	1.0 mg kg^{-1}	Anglerfish, Atlantic catfish, bonito, eel, emperor, orange roughy, rosy soldierfish, grenadier, halibut, kingklip, marlin, megrim, mullet, pink cusk eel, pike, plain bonito, poor cod, Portuguese dogfish, rays, redfish, sail fish, scabbard fish, seabream, pandora, shark (all species), snake mackerel or butterfish, sturgeon, swordfish, tuna.
EC/1881/2006	Benzo[*a*]pyrene	5.0 µg kg^{-1}	Smoked fish and fishery products
EC/1881/2006	Benzo[*a*]pyrene	5.0 µg kg^{-1}	Crustacea & cephalopods, other than smoked and excluding crab brown meat, head / thorax meat of lobster (and similar spp.)
EC/1881/2006	Benzo[*a*]pyrene	5.0 µg kg^{-1}	Bivalve molluscs
EC/1259/2011	Dioxins & furans[2]	3.5 pg g^{-1} TEQ	Fish muscle and fishery products, excluding eel and freshwater fish

EC/1259/2011	Dioxins, furans & DL-PCBs[1]	6.5 pg g^{-1} TEQ	Fish muscle and fishery products, excluding eel and freshwater fish
EC/1259/2011	ICES 6 PCBs	75 µg kg^{-1}	Fish and fishery products, excluding eel and freshwater fish
EC/1259/2011	Dioxins & DL-PCBs	20 pg g^{-1} TEQ	Fish liver and derived products, with the exception of marine oils
EC/1259/2011	ICES 6 PCBs	200 µg kg^{-1}	Fish liver and derived products, with the exception of marine oils

[1]Individual compounds as listed in EC Regulation EC/1881/2006.

Statistical Analysis

The concentrations of Hg, Cd, Pb and ICES6 PCBs in each species and area were compared against the regulatory limits by assuming they were log-normally distributed, estimating the 95th percentile of the distribution, and testing whether this was significantly below the regulatory limit at the 5% significance level.

Formally, let y_i, i = 1…n, be the n measured concentrations of a particular element or compound in a particular species and area. Further, assume that the concentrations have a log-normal distribution:

$$y \sim \log N(\mu, \sigma^2)$$

where µ and σ are the mean and standard deviation of log-concentration. Assuming for now that no concentrations are below the limit of detection (LoD), the likelihood of the data is given by:

$$L(\mu, \sigma; y) = \prod_{i=1}^{n} \frac{1}{\sigma} \varphi\left(\frac{\log y_i - \mu}{\sigma} \right)$$

where φ is the probability density function of a standard normal distribution. The maximum likelihood estimators of µ and σ are then:

$$\hat{\mu} = \frac{1}{n} \sum_{i=1}^{n} \log y_i$$

$$\hat{\sigma}^2 = \frac{1}{n} \sum_{i=1}^{n} (\log y_i - \hat{\mu})^2$$

The maximum likelihood estimator of the 100ρ percentile of the concentration distribution is then given by:

$$\hat{q}_{100\,\rho} = \exp\left(\hat{\mu} + \hat{\sigma}\,\Phi^{-1}(\rho)\right)$$

where Φ is the cumulative distribution function of a standard normal distribution. In particular, the estimator of the median concentration is:

$$\hat{q}_{50} = \exp\left(\hat{\mu} + \hat{\sigma}\Phi^{-1}(0.5)\right) = \exp(\hat{\mu})$$

and of the 95th percentile is:

$$\hat{q}_{95} = \exp\left(\hat{\mu} + \hat{\sigma}\Phi^{-1}(0.95)\right) \cong \exp(\hat{\mu} + 1.645\,\sigma)$$

Profile likelihood methods can be used to obtain an upper likelihood limit on q_{95}. The upper limit can then be used to test whether q_{95} is below the regulatory level. To obtain the profile likelihood for q_{95}, it is necessary to rewrite the likelihood in terms of μ and q_{95} as:

$$L(\mu, q_{95}; y) = \prod_{i=1}^{n} \frac{\Phi^{-1}(0.95)}{\log q_{95} - \mu} \varphi\left(\frac{\Phi^{-1}(0.95)(\log y_i - \mu)}{\log q_{95} - \mu}\right)$$

The profile likelihood for q_{95} is then $L(\hat{\mu}(q_{95}), q_{95}; y)$, where $\hat{\mu}(q_{95})$ is the value of μ that maximises $L(\mu, q_{95}; y)$ given q_{95}. Let $X^2_{\text{crit}}(\alpha)$ be the 'critical' value that satisfies:

$$\Pr(X_1^2 \le X^2_{\text{crit}}(\alpha)) = 1 - 2\alpha$$

Where X_1^2 is a X^2 distribution on one degree of freedom and Pr is the probability. Then an approximate one-sided upper $100(1-\alpha)\%$ likelihood limit for q_{95} is the value of q_{95} that satisfies:

$$2(\log L(\hat{\mu}, \hat{q}_{95}; y) - \log L(\hat{\mu}(q_{95}), q_{95}; y)) = X^2_{\text{crit}}(\alpha)$$

For example, a one-sided upper 95% likelihood limit for q_{95} is obtained by setting α to 0.05 which gives $X^2_{\text{crit}}(\alpha) = 2.706$. In practice, the upper likelihood limit is found numerically. If the upper likelihood limit is less than the regulatory level, then it can be concluded that q_{95} is less than the regulatory level at the 100α% significance level.

The likelihood limits are only approximate, with the approximation improving as *n* (the number of concentration measurements) increases. For the target sample size of 20, simulations suggest that α = 0.05 gives a coverage of only about 92%, so the

upper likelihood limit is too low. This means that a test at the nominal 5% significance level is actually at the 8% level, so we are more likely to conclude that the q_{95} is below the regulatory level than we should. To get a test which is actually at the 5% level, simulations show that it is necessary to use a nominal 3% significance level by setting $\alpha = 0.03$.

Adjustments to the likelihood are necessary if some of the concentrations are below the LoD. In the case of the ICES6 PCBs, this is taken to mean that any of the individual PCBs is below the LoD. Suppose the first m measurements are below the LoD and the rest are above. Further, let y_i, $i = 1 \ldots m$, and z_i, $i = 1 \ldots m$, denote the largest and smallest possible values the concentration measurements could take. For Hg, Cd and Pb, the y_i are the LoDs and the z_i are zero. For the ICES6 PCBs, it depends on how many of the individual PCBs are below the LoD, but essentially the y_i are the sum of the LoDs (if below) and the measured concentrations (if not) and the z_i are the sum of zeros (if below) and the measured concentrations (if not). The likelihood is then:

$$L(\mu, \sigma; y) = \left\{ \prod_{i=1}^{m} \left(\Phi\left(\frac{\log y_i - \mu}{\sigma} \right) - \Phi\left(\frac{\log z_i - \mu}{\sigma} \right) \right) \right\} \left\{ \prod_{i=m+1}^{n} \frac{1}{\sigma} \varphi\left(\frac{\log y_i - \mu}{\sigma} \right) \right\}$$

The rest of the methods then follow accordingly, although the maximum likelihood estimators of μ and σ now have to be found numerically. Simulations show that when $n = 20$ and the proportion of measurements below the detection limit is 60%, setting $\alpha = 0.03$ still gives a test at the desired 5% significance level.

The methods above break down when most of the measurements are below the LoD. In particular, when all the measurements are below the LoD, the maximum likelihood estimator of μ is undefined. A pragmatic solution is to assume that σ (standard deviation of log concentration) is large and known and to adjust the methods accordingly. With σ assumed known, the maximum likelihood estimator of μ and q_{95} is defined and an upper likelihood limit on q_{95} can be found. Taking a large value of σ is a precautionary measure, ensuring that the upper likelihood limit on q_{95} is, if anything, too high. We have used $\sigma = 1$, which is greater than any of the estimates of σ for data sets with most measurements above the detection limit . A simpler alternative would be to use a non-parametric sign test to test whether q_{95} is less than the regulatory level. This would be applicable no matter how many concentrations are below the LoD. However, at least 60 concentration measurements would be required to give a test at the 5% significance level.

Results and Discussion

Implementation of Sampling Designs

It proved difficult to implement the sampling designs successfully, despite their seeming simplicity. Bad weather, a lack of fish in key sampling strata, and communication and logistic issues all contributed. For example, Table 2 shows the target number of samples for North Sea haddock, the number of contingency samples (in case the target number was not caught in some strata) and the actual number of fish sampled. Haddock were only sampled in the more northerly part of the survey area (Table 2), despite marketable haddock being caught in the southern part. Given that the sampling design had been so compromised, no attempt was made to sub-sample the contingency fish to achieve the target sample size of 20 fish (sampled with probability proportional to landings). Instead, all 22 fish were chemically analysed and used to estimate q_{95}. However, the estimates of q_{95} should be interpreted as characterising the distribution of concentrations in haddock in the more northerly part of the survey area.

Table 2

Implementation of the sampling design for North Sea haddock using the Q1 bottom trawl survey.

Statistical rectangle	Target samples	Contingency samples	Actual samples
50 E7	1		
50 E8	1		1
49 E6		1	
49 E8	1	1	2
49 E9	3	4	8
48 E6	1		
48 E8	1		1
48 E9	3	4	7
47 E8	1		1
46 E8	1	1	2
45 E7	1	1	
44 E7		2	
44 E8	2	1	
44 E9	2	1	
44 F0	2	3	
43 E9		1	

Few North Sea monkfish were caught during the Q3 bottom trawl survey and only four monkfish were sampled and analysed for D9 purposes (Table 3). Although 26 herring were sampled, none were taken from the key strata in the centre of the survey area (47 E8, 47 E9, 46 E7, 46 E8) despite marketable fish being caught. Again, given that the sampling design had been compromised, no attempt was made to sub-sample the contingency fish to achieve the target sample size, and 26 fish were chemically analysed and used to estimate q_{95}.

Table 3

Implementation of the sampling design for North Sea monkfish and herring using the Q3 bottom trawl survey.

Statistical rectangle	Monkfish samples			Herring samples		
	target	in case	actual	target	in case	actual
52 E9	1	2				
51 E8	2					
51 E9	1					
50 E7	1					
50 E8		2				
50 E9	3				3	2
50 F0		2			1	1
50 F1					1	
49 E6	1	7	2			
49 E7	1			1		1
49 E8					2	1
49 E9				1	2	
48 E6	4	1	1		1	2
48 E7		1	1		1	3
48 E8				2	3	4
48 E9	1					
48 F0	3	1		1		1
47 E7				1		1
47 E8				4	4	
47 E9				1	2	
46 E6					1	1
46 E7				3	3	
46 E8				2		
46 E9				1		1
46 F0	1	2			2	2
46 F1				1		1
45 E9		1			1	1
44 E8					1	1
44 F0		1		1		
44 F1	1				1	1
43 F0				1	1	2

West Coast sampling was more straightforward, partly because there were fewer strata, with multiple hauls in each. Table 4 shows the target sample size and the actual number of samples analysed for D9 purposes. The haddock were actually sampled on both the 2013 Q4 bottom trawl survey (which was not completed due to bad weather) and the 2014 Q1 bottom trawl survey, and the slight mismatch between the target and the realised sample allocation arose because the strata for the two surveys are not quite the same. The monkfish all came from the Q1 bottom trawl survey when a lack of fish resulted in only 16 samples being taken. Herring were sampled on the Q3 herring acoustic survey, but fish were only sampled from one haul and so were not suitable for D9 purposes.

Table 4

Implementation of the sampling design for West Coast haddock and monkfish using the Q1 bottom trawl survey.

Stratum	Haddock samples		Monkfish samples	
	target	actual	target	actual
R1	2	3	4	6
R2			3	2
R3	1	1		
G1	16	15	11	8
LB	1	1	2	

Estimates of q_{95}

The concentrations of the ICES6 PCBs, mercury, cadmium and lead measured in each fish are shown in Figure 2 and summarised in Table 5. All concentrations were below the regulatory level. There were detectable levels of mercury in all fish, but most cadmium and lead concentrations were below the LoD. All but one herring had detectable levels of at least one of the ICES6 PCBs. However, few haddock and monkfish had detectable levels of any of the ICES6 PCBs. This will be due, in part, to the lower lipid content of haddock and monkfish muscle.

The estimates of q_{95} with their upper 95% likelihood limits are also shown in Figure 2 and given in Table 5. All but one of the q_{95}s are significantly below the regulatory level at the 5% significance level. The exception is mercury in monkfish on the West Coast, where the point estimate of q_{95} is about half the regulatory level, but the upper 95% likelihood limit is just above. Only 16 monkfish were sampled on the West Coast (the target sample size was 20), so the estimate of q_{95} is not as precise as intended. Monkfish has a low lipid content (ranging from 0.48% to 0.64% in this study) but has the highest trophic level of the three species sampled, with a trophic

level of 4.5, compared to 4.1 for haddock and 3.2 for herring[9]. Although the q_{95} for mercury in monkfish in the North Sea is significantly below the regulatory level, this result is based on only four fish. These fish were all that were available, but do not represent the target population (Table 3), so further sampling is required to provide a stronger evidence base for D9 purposes.

Table 6 gives estimates of q_{95} for a selection of other metals (with no regulatory levels).

Table 5

For each contaminant, species and area (NS = North Sea, WC = West Coast), the number of samples with concentrations above the LoD, below the LoD, or, in the case of the ICES6 PCBS, with a mix of congeners above and below the LoD; the estimates of the standard deviation of log concentration σ, the median concentration q_{50}, the 95th percentile of the concentration distribution q_{95} and the upper 95% likelihood limit on q_{95}; and the regulatory level. All concentration units are µg kg^{-1}. A * indicates that there were insufficient samples above the LoD to estimate q_{50} and σ jointly, so σ has been fixed at the 'high' value of one. In these cases, the point estimates of q_{50} and q_{95} should be treated with caution, but the upper limits do give a reasonable upper bound on plausible values of q_{95}.

Contaminant	Species	Area	Number of samples			σ	q_{50}	q_{95}	Upper limit	Reg. level
			>LoD	<LoD	Mix					
ICES6 PCBs	Herring	NS	10	1	15	0.68	3.0	10.9	17.2	75
	Monkfish	NS	0	4	0	1*	0	0	2.6	75
	Monkfish	WC	0	15	1	1*	0.1	0.9	1.9	75
	Haddock	NS	0	21	1	1*	0.1	0.9	1.7	75
	Haddock	WC	0	19	1	1*	0.1	0.8	1.7	75
Mercury	Herring	NS	26	0		0.47	45	107	144	500
	Monkfish	NS	4	0		0.40	115	245	630	1000
	Monkfish	WC	16	0		0.78	119	520	1025	1000
	Haddock	NS	22	0		0.48	58	143	202	500
	Haddock	WC	20	0		0.32	100	180	228	500
Lead	Herring	NS	1	25		1*	2.5	16.3	30.6	300
	Monkfish	NS	0	4		1*	0	0	50.8	300
	Monkfish	WC	0	16		1*	0	0	22.0	300
	Haddock	NS	1	21		1*	2.3	16.3	32.0	300
	Haddock	WC	4	16		1*	5.7	37.1	60.6	300
Cadmium	Herring	NS	5	21		1*	1.7	11.5	17.9	50
	Monkfish	NS	0	4		1*	0	0	17.8	50
	Monkfish	WC	0	16		1*	0	0	7.7	50
	Haddock	NS	0	22		1*	0	0	6.6	50
	Haddock	WC	0	20		1*	0	0	6.9	50

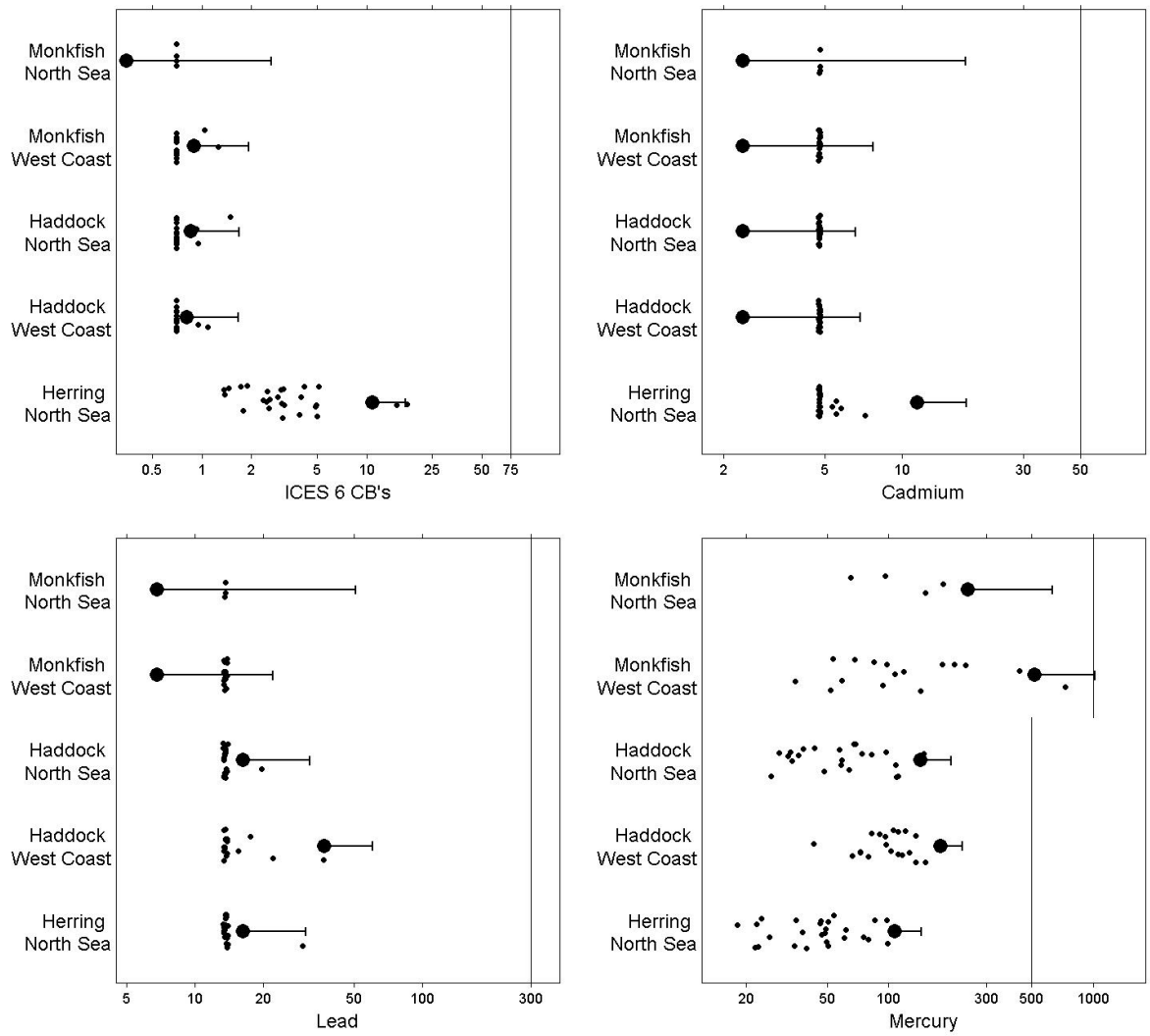

Figure 2: The concentration measurements (small dots), estimates of q_{95} (large dots), with upper 95% likelihood limits (small vertical lines) and the regulatory levels (long vertical lines). The concentration measurements have been jittered to avoid overlap. Estimates of q_{95} equal to zero have been plotted at half the LoD to avoid distorting the Figure.

Table 6

Estimates of q_{95} with corresponding upper 95% likelihood limits for selected metals.

Contaminant	Species	Area	Samples >LoD	Samples <LoD	σ	q_{50}	q_{95}	Upper limit
Manganese	Herring	NS	26	0	0.37	237	477	606
	Monkfish	NS	4	0	0.23	76	117	201
	Monkfish	WC	16	0	0.34	83	166	229
	Haddock	NS	22	0	0.15	89	118	132
	Haddock	WC	20	0	0.32	89	162	206
Chromium	Herring	NS	5	21	1*	14.1	92.3	141.2
	Monkfish	NS	0	4	1*	0	0	114.4
	Monkfish	WC	1	15	1*	7.0	45.6	92.1
	Haddock	NS	0	22	1*	0	0	42.3
	Haddock	WC	3	17	1*	11.6	76.3	127.4
Iron	Herring	NS	26	0	0.40	6811	14361	18528
	Monkfish	NS	4	0	0.14	1354	1757	2428
	Monkfish	WC	12	4	0.45	998	2320	3614
	Haddock	NS	20	2	0.43	1066	2399	3298
	Haddock	WC	19	1	0.51	1273	3354	4985
Copper	Herring	NS	26	0	0.40	592	1259	1630
	Monkfish	NS	4	0	0.40	132	282	721
	Monkfish	WC	13	3	0.37	123	249	356
	Haddock	NS	22	0	0.19	120	171	196
	Haddock	WC	20	0	0.36	143	283	371
Silver	Herring	NS	0	16	1*	0	0	8.0
	Monkfish	NS	0	4	1*	0	0	18.4
	Monkfish	WC	0	15	1*	0	0	8.3
	Haddock	NS	11	11	0.61	5.3	16.7	29.8
	Haddock	WC	4	15	1*	2.0	13.0	21.6
Cobalt	Herring	NS	21	5	1*	2.0	13.1	20.4
	Monkfish	NS	0	4	1*	0	0	20.4
	Monkfish	WC	0	16	1*	0	0	8.8
	Haddock	NS	0	22	1*	0	0	7.5
	Haddock	WC	1	19	1*	1.0	6.3	12.8
Arsenic	Herring	NS	26	0	0.33	1421	2626	3238
	Monkfish	NS	4	0	0.61	7983	25158	104887
	Monkfish	WC	16	0	0.48	8271	20284	30651
	Haddock	NS	22	0	0.31	10462	18701	23288
	Haddock	WC	20	0	0.57	5592	16251	24911
Vanadium	Herring	NS	16	0	0.44	62.3	142.5	206.4
	Monkfish	NS	4	0	0.17	87.6	119.5	175.8
	Monkfish	WC	5	11	1*	9.6	62.9	102.4
	Haddock	NS	0	22	1*	0	0	19.7
	Haddock	WC	2	18	1*	4.6	30.3	52.5

TEQs for Dioxins and Dioxin-Like CBs

Due to the expense of high resolution GC-MS, methods have been proposed for predicting the total TEQs for chlorinated dioxins and 'dioxin-like' CBs in fish tissue using total PCB concentrations or indicator PCBs. In summary, Bhavsar *et al.* proposed that the total PCB concentration (the sum of 159 of the possible 209 congeners) could be used to predict the total TEQ for 'dioxin-like' PCBs[7] using the relationship:

$$TEQ_{DL\text{-}est} = 2.56 \times 10^{-3} \times \text{Total PCB concentration}$$

Lasrado *et al.* looked at four models for predicting TEQs using the US Environment Protection Agency fish tissue study[8]. They concluded that the analysis of selected PCBs could be used to estimate total TEQs from chlorinated dioxins, furans and DL-PCBs and proposed the following model using indicator PCBs:

$$TEQ_{est} = 0.95 + 0.21[CB138] - 0.08[CB153] + 0.27[CB118]$$

Concentration units for TEQs are pg g^{-1} wet weight and for the PCBs µg kg^{-1} wet weight.

$TEQ_{DL\text{-}est}$ and TEQ_{est} were estimated for each fish sampled in this study (with $TEQ_{DL\text{-}est}$ based on the sum of all the 28 PCB congeners measured). TEQ_{est} was always greater than $TEQ_{DL\text{-}est}$ and always less than the European Commission's maximum level of 6.5 pg g^{-1} wet weight.

Lessons Learned and Future Sampling Requirements

The aim of this Descriptor 9 Sampling Programme was to address the requirements of Descriptor 9 with minimal extra cost by making use of existing cruises for collection of samples. Fish stock assessment surveys were the obvious choice to base the sampling programme on as they covered both the North Sea and West Coast and would be sampling all three target species. However, obtaining the required samples on these surveys proved to be more difficult than expected. Despite having contingency sampling locations the target number of fish for each species and area were not always achieved. This was partly due to poor weather and the lack of fish in some areas, but may have also been due to the complexity of the sampling design and relying on staff not directly involved in the project. The West Coast sampling designs were easier to implement than those in the North Sea because they had larger strata, with several hauls in each. Future North Sea

sampling would be simplified by amalgamating the statistical rectangles (used as strata in the designs reported on here) into larger strata (say, with four rectangles in each).

From the data presented here, it is likely that GES would be achieved with the possible exception of mercury in monkfish. On the West Coast, the point estimate of q_{95} was about half the regulatory level but, taking a precautionary approach, it is not possible to say that the q_{95} was significantly below the regulatory level. In the North Sea, the q_{95} was significantly below the regulatory level, but was based on only four fish which do not adequately represent the target sampling population. Additional monkfish samples have already been collected from the North Sea, which will hopefully improve this component of the assessment. For Cd, Pb and PCBs (and possibly dioxins) it may be sufficient to undertake sampling and analysis for Descriptor 9 every six years to confirm that there have been no changes and concentrations are still below the regulatory limits.

Conclusions

1. For the Marine Strategy Framework Directive's Descriptor 9 contaminant concentrations in fish and seafood should be compared against the EC regulatory levels. European regulatory levels are available for trace metals (Cd, Hg and Pb), dioxins, DL-PCBs and non DL-PCBs (ICES6, CB28, 52, 101, 138, 153 and 180) in fish muscle, crustacea and bivalve molluscs, for PAHs (benzo[*a*]pyrene), in crustacea and bivalves and for dioxins (including DL-PCBs) and non DL-PCBs in fish liver (EC/1881/2006 and EC/1259/2011).

2. Although data from Scottish shellfish monitoring programmes will be suitable for Descriptor 9 assessments, data from current fish monitoring programmes will be of little use. For environmental fish monitoring programmes commercially exploited fish species/size-ranges are not targeted and, with the exception of metals, contaminants are measured in fish liver rather than in the edible flesh.

3. A fish sampling programme for Descriptor 9 was designed in 2013/14. Haddock, monkfish and herring were selected based on their importance to the human diet (based on landings data) and their ability to accumulate contaminants. Fish were sampled from existing research surveys for obtaining indices of abundance for use in fish stock assessments in 2013/2014 from haul locations with probabilities proportional to landings.

4. There were some difficulties implementing the sampling programme, with bad weather, lack of fish in key strata and communication and logistical problems meaning that the target number of fish were often not sampled. Future North Sea surveys would be simplified by reducing the number of sampling strata, and having several hauls in each.

5. The edible muscle tissue of all samples were analysed for PCBs and trace metals. As expected PCBs were found at higher concentrations in the herring due to its high lipid content, and were below the LoDs in nearly all haddock and monkfish.

6. Cd and Pb were mainly below detection limits in all three species whilst Hg exceeded the LoD in all samples. Hg was higher in the monkfish which has a low lipid content but the highest trophic level.

7. EC maximum limits were not exceeded in any individual sample for trace metals or PCBs. The 95th percentiles of the distributions of trace metal and ICES6 PCBs concentrations were estimated for each species and area. These were significantly below the regulatory levels, apart from Hg in monkfish on the West Coast, where the point estimate of the 95th percentile was about half the regulatory level, but the upper likelihood limit was above it. However, only 16 monkfish were sampled and analysed on the West Coast, rather than the target of 20, so the estimate of the 95th percentile was not as precise as intended. In the North Sea, the 95th percentile of the Hg distribution in monkfish was significantly below the regulatory level, but was based on only four fish which did not adequately represent the target sampling population.

8. A regulatory level is available for dioxins, but MSS does not have the capability to measure dioxins. Therefore, dioxin TEQs were estimated from the PCB concentrations using published models. All were below the EC maximum limit.

9. Based on these results, it is likely that GES would be achieved for Descriptor 9 in both the North Sea and West Coast of Scotland, with the possible exception of Hg in monkfish. Additional analysis is planned for Hg in monkfish to improve the assessment.

10. For PCBs, Cd and Pb, it may be sufficient to sample every six years to confirm concentrations are below regulatory levels.

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